The method and means of accomplishing each of the above objectives as well as others will become apparent from the detailed description of the invention which follows hereafter.

SUMMARY OF THE INVENTION

The present invention describes a method of producing phosphatidylserine (PS) that has several unique aspects. The method is more efficient and provides better transphosphatidylation activity in comparison to previous PS production methods.

The method first involves producing phospholipase D (PLD) enzyme from phospholipase-producing microorganisms. The PLD is produced in an optimized growth media that preferably includes *Streptomyces cinnamoneum*, and most preferably includes ATCC 11894. The enzyme is then harvested, filtered, and concentrated, and preferably stabilized through the use of a chelating agent.

The PLD is next reacted with a lecithin and racemic or enantiomerically pure serine, preferably L-serine, and a lower (C_1-C_5) alcohol to produce PS. To accomplish this, the phosphatide is dissolved in one or more organic solvents. Serine, a metal, and buffer that is preferably 20-50 mM sodium acetate are added to the PLD. The organic and aqueous phases are combined, a lower alcohol (C_1-C_5) is added to create the aqueous/organic interphase wherein the transphosphatidylation reaction occurs, and the diphasic mixture is then reacted, preferably with rapid stirring, and at a preferred temperature and time of 32 ± 1°C and for 18±4 hours, respectively.

The present invention differs from previous methods in that it incorporates a chelating agent and calcium or other metal to selectively enhance the transphosphatidylation activity of PLD. Further, the present method is the first to use a combination of organic solvent and a lower (C_1-C_5) alcohol during the transphosphatidylation reaction in order to create an appropriate aqueous and organic interphase and maximize PS production. In addition, the method is the first known to reuse the PLD enzyme and serine up to five times in the production reaction, thereby increasing the efficiency of PS production. The method is also the first known to reclaim the serine using an ethanol fractionation of the serine. A preferred embodiment of the invention